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# Operation of a Two-stage Continuous Fermentation Process Producing Hydrogen and Methane from Artificial Food Wastes

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**Abstract**

An anaerobic two-stage continuous fermentation process with combined thermophilic hydrogenogenic and methanogenic stages (two-stage fermentation process) was applied to artificial food wastes on a laboratory scale. In this report, organic loading rate (OLR) conditions for hydrogen fermentation were optimized before operating the two-stage fermentation process. The OLR was set at 11.2, 24.3, 35.2, 45.6, 56.1, and 67.3 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$ with a temperature of 60°C, pH5.5 and 5.0% total solids. As a result, approximately 1.8-2.0 mol–$H_2$ mol-$hexose^{-1}$ was obtained at the OLR of 11.2-56.1 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$. In contrast, it was inferred that the hydrogen yield at the OLR of 67.3 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$ decreased because of an increase in lactate concentration in the culture medium. The performance of the two-stage fermentation process was also evaluated over three months. The hydraulic retention time (HRT) of methane fermentation was able to be shortened 5.0 days (under OLR 12.4 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$ conditions) when the OLR of hydrogen fermentation was 44.0 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$, and the average gasification efficiency of the two-stage fermentation process was 81% at the time.

## 1 Introduction

Global warming caused by increasing concentrations of carbon dioxide and other greenhouse gases has led to interest in renewable energy such as solar, wind, and biomass energy. Furthermore, although hydrogen is a secondary energy, it is well known as a clean energy which does not emit carbon dioxide itself. The future popularization of these energy sources is essential to preventing global climate change. For the exploitation of biomass energy, and especially for fermentation systems, it is desirable to downsize the fermentation reactor and improve the raw materials energy recovery efficiency. A two-stage continuous fermentation process composed of a hydrogenogenic stage in the hydrogen fermentation reactor followed by a methanogenic stage in the methane fermentation reactor (two-stage fermentation process) has been researched to solve these issues [1-3]. Theoretically, this process has the potential to improve the energy recovery efficiency up to approximately 9%, compared with a single-stage process, when glucose is used as the raw material [4]. Moreover, the methane production rate in the two-stage fermentation process is supposed to increase due to the production of volatile fatty acids (VFAs) such as acetate and butyrate in the hydrogen fermentation process. In this report, optimum organic loading rate (OLR) conditions for the hydrogenogenic process were evaluated using thermophilic microflora mainly composed of *Clostridium* species screened through approximately 100 types of soil. Then, a two-stage fermentation process applying this hydrogen fermentation was conducted to evaluate the influence of the OLRs used in methane fermentation on methane production capacity.

## 2 Material and Methods

### 2.1 Feed material and seed organism

Artificial food wastes (AFW) composed of vegetables, chicken, fish and rice in wet-weight proportions of 87%, 3%, 3% and 7%, respectively, were prepared as a culture medium for hydrogen fermentation ($H_2$ fermentation). The AFW was then diluted with water to obtain a total solids concentration of 5%. The average amount of dichromate chemical oxygen demand ($COD_{cr}$) of the culture medium after water dilution was 62,350 mg $L^{-1}$. Thermophilic microflora mainly composed of *Clostridium* species, screened through approximately 100 types of soil were used for the hydrogenogenic process. An anaerobic digestion sludge obtained from a sewage treatment plant was used as the seed organism of the methane fermentation ($CH_4$ fermentation).

### 2.2 Experimental procedure

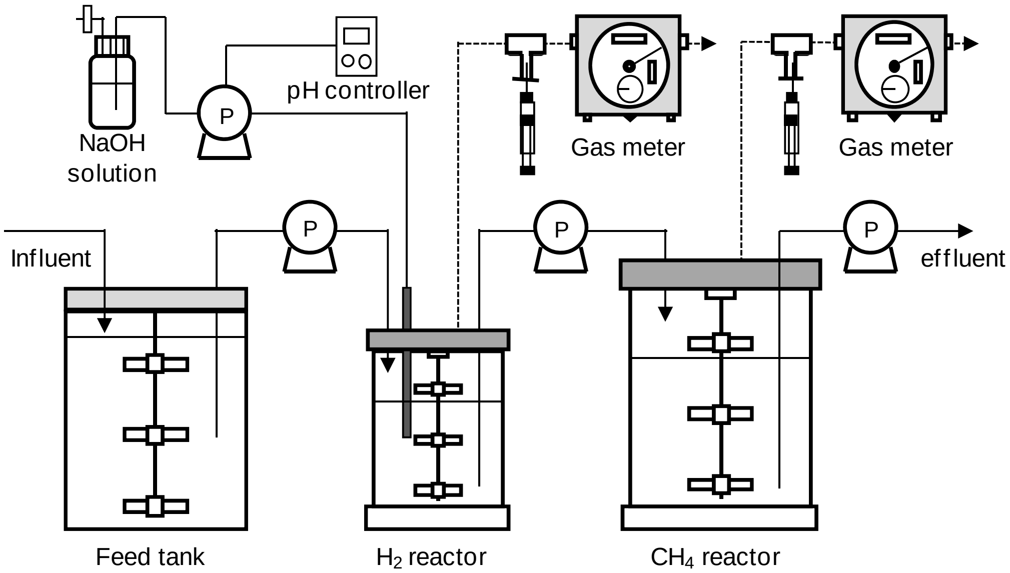


**Figure 1: Schematic diagram of two-stage fermentation process (solid and dotted lines indicate the flow of raw material and product biogas, respectively).**

A schematic diagram of the experimental apparatus for the two-stage fermentation process is shown in Figure 1. In the experiment, the food waste slurry was fed into a fermentation hydrogen reactor ($H_2$ reactor) with a 0.8 L effective volume (mini-jar fermentor TS-A1 (1L); Takasugi Corporation). The residue from the $H_2$ reactor was fed into a fermentation methane reactor ($CH_4$ reactor) with a 2.5 L effective volume (mini-jar fermentor TS-A3 (3L)). The effluent whose quantity was the same as the amount of residue supplied from the $H_2$ reactor was discharged from the $CH_4$ reactor. Although the pH of the culture medium in the $H_2$

reactor was regulated at 5.5 by automatic titration of a NaOH solution, the culture medium in the $CH_4$ reactor was not regulated. Electric heating apparatuses installed at the bottom of the reactor were automatically controlled to maintain the operational temperatures of the hydrogenogenic and methanogenic processes at 60°C and 55°C, respectively. All roller pumps, except for the pH regulation pump, were activated every four hours, and the working time of each pump was changed according to experimental conditions.

### 2.3 Methods of analysis

The amount of gas produced in the $H_2$ and $CH_4$ reactors was measured with a wet-gas meter (W-NK-0.5; Shinagawa Corporation). The proportions of hydrogen ($H_2$), methane ($CH_4$), nitrogen ($N_2$) and carbon dioxide ($CO_2$) in the biogas were measured with a micro-gas chromatograph (CP-4900; Varian Technologies Japan, Ltd.) equipped with a thermal conductivity detector and two columns. $H_2$ and $CH_4$ were analyzed using a 10-m stainless-steel column packed with Molsieve 5A. Injector, detector and column temperatures were kept at 170°C, 100°C and 100°C, respectively. $CO_2$ was analyzed using a 10-m stainless-steel column packed with PoraPLOT Q. Injector, detector and column temperatures were kept at 190°C, 80°C and 80°C, respectively.

The concentrations of lactate, acetate, propionate, n-butyrate and n-valeriate were determined with an ion chromatograph (ICS-1000; DIONEX Corporation) equipped with an Ion Pac ICE-AS1 analytical column, an AMMS-ICE-300 suppressor and an ICS-1000 conductivity detector. One mM octanesulfonate was used as the eluent (flow rate of 1.0 mL $min^{-1}$) and the detector temperature was set at 35°C.

The $COD_{cr}$ was determined using a portable spectrophotometer (DR2800; HACH COMPANY) and HACH1076 as a reagent.

## 3 Results and Discussion

### 3.1 The performance of $H_2$ fermentation

The $H_2$ fermentation was optimized prior to carrying out the two-stage fermentation process experiment. Throughout the experiment, the composition of the product biogas was 50-52% $H_2$ and 48-50% $CO_2$, and no $CH_4$ was detected. Table 1 shows the results, which are calculated as average values, summarizing the effects of the OLR on $H_2$ production capacity and the concentration of VFAs in the hydrogenogenic process.

**Table 1: Hydrogen production capacity and VFA concentration in the hydrogenogenic process.**

| OLR | HRT | $H_2$ yield | | $H_2$ production rate | concentration of VFAs (mg $L^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| (g-$COD_{cr}$ $L^{-1}day^{-1}$) | (day) | (L $kg_{AFW}^{-1}$) | (mol-$H_2$ mol-hexose$^{-1}$) | (L $L^{-1}day^{-1}$) | lactate | acetate | propionate | n-butyrate |
| 11.2 | 5.6 | 31.2 | 1.9 | 1.8 | 363 | 3049 | 37 | 10451 |
| 24.3 | 2.6 | 30.5 | 1.8 | 3.1 | 323 | 3956 | 45 | 9214 |
| 35.2 | 1.8 | 33.2 | 2.0 | 5.1 | 172 | 4648 | 54 | 10220 |
| 45.6 | 1.4 | 33.1 | 2.0 | 6.5 | 241 | 4863 | 47 | 11064 |
| 56.1 | 1.1 | 32.7 | 1.9 | 8.4 | 324 | 4881 | 28 | 9628 |
| 67.3 | 0.9 | 27.5 | 1.6 | 8.3 | 1748 | 4703 | 17 | 9973 |

The $H_2$ production rate increased along with the OLR up to 56.1 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$ with a hydraulic retention time (HRT) of 1.1 days. The $H_2$ yield was 1.8-2.0 mol-$H_2$ mol-$hexose^{-1}$ under these conditions. However, the $H_2$ production rate decreased at the OLR of 67.3 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$ (HRT of 0.9 days), compared with the $H_2$ production rate at the OLR of 56.1 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$ (HRT of 1.1 days). An increase in the concentration of lactate was observed at the OLR of 67.3 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$. It is known that, in anaerobic digestion, VFAs are produced as shown in Table 2. Since there is no $H_2$ production accompanying lactate production, it was inferred that the $H_2$ production capacity at the OLR of 67.3 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$ decreased because of an increase in lactate concentration in the $H_2$ fermentation culture medium. In addition, lactate bacteria have been reported to be inhibitory to $H_2$ fermentation [5].

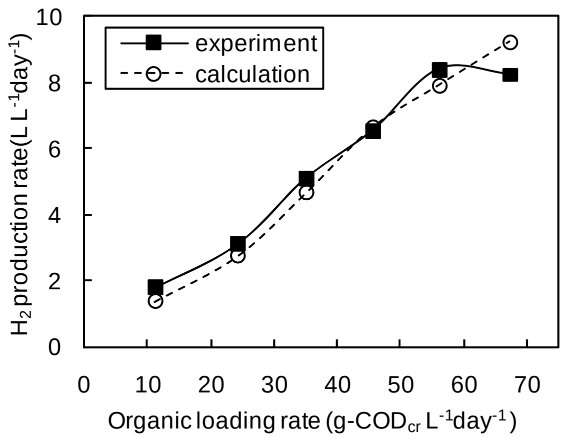


**Figure 2: Comparison of experimental and theoretical value for $H_2$ production rate.**

For further examination of the performance of $H_2$ fermentation, the $H_2$ production rate under each condition was compared with the theoretical $H_2$ production rate. The theoretical value was calculated from the concentration of VFAs in the $H_2$ reactor, assuming that the stoichiometry of $H_2$ production and consumption was as shown in Table 2 (a), (d) and (e) [6]. Figure 2 shows the comparison of experimental and theoretical values. The results show that the experimental values up to the OLR of 56.1 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$ are close to the theoretical values. $H_2$ can therefore be expected to be produced effectively under these conditions. At the OLR of 67.3 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$, on the other hand, the experimental value is lower than the theoretical value. From this, it is inferred that, besides reaction (a), different reactions such as those in Table 2 (b) and (c) occurred to produce acetate, resulting in a decrease in the $H_2$ production rate. In addition, although the production of lactate is not a factor in the calculation, an increase in the lactic acid concentration is observed. This therefore suggests that the fermentation pattern is affected by an increase in the amount of lactate in the culture medium.

**Table 2: VFA production with anaerobic digestion.**

| Products | Chemical reaction | |
|---|---|---|
| acetate | $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ | (a) |
| | $C_6H_{12}O_6 \rightarrow 3CH_3COOH$ | (b) |
| | $4H_2 + 2CO_2 \rightarrow CH_3COOH$ | (c) |
| n-butyrate | $C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$ | (d) |
| propionate | $CH_3COCOOH + 2H_2 \rightarrow CH_3CH_2COOH + H_2O$ | (e) |
| lactate | $C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH$ | (f) |

### 3.2 Evaluation of performance of two-stage fermentation process with combined $H_2$ and $CH_4$ reactors

The two-stage fermentation process was evaluated over a period of more than three months. Figure 3 shows the time course for the $H_2$ and $CH_4$ production rates. Initial operation data are not shown because of unstable experimental conditions. The OLR used in the $H_2$ fermentation was set between 30.5-54 g-COD$_{cr}$ L$^{-1}$ day$^{-1}$ in the experiment. On day 57, all the culture medium in the $H_2$ reactor was replaced with a new culture since a sufficient amount of $H_2$ could not be obtained due to feed putrefaction. After $H_2$ fermentation was restarted, $H_2$ production stabilized around 31.0 L kg$_{AFW}$$^{-1}$, which is equivalent to 1.8 mol-$H_2$ mol-hexose$^{-1}$. The composition of the biogas was 50-55 % $H_2$ and 45-50% $CO_2$, and no $CH_4$ was detected in the $H_2$ reactor.

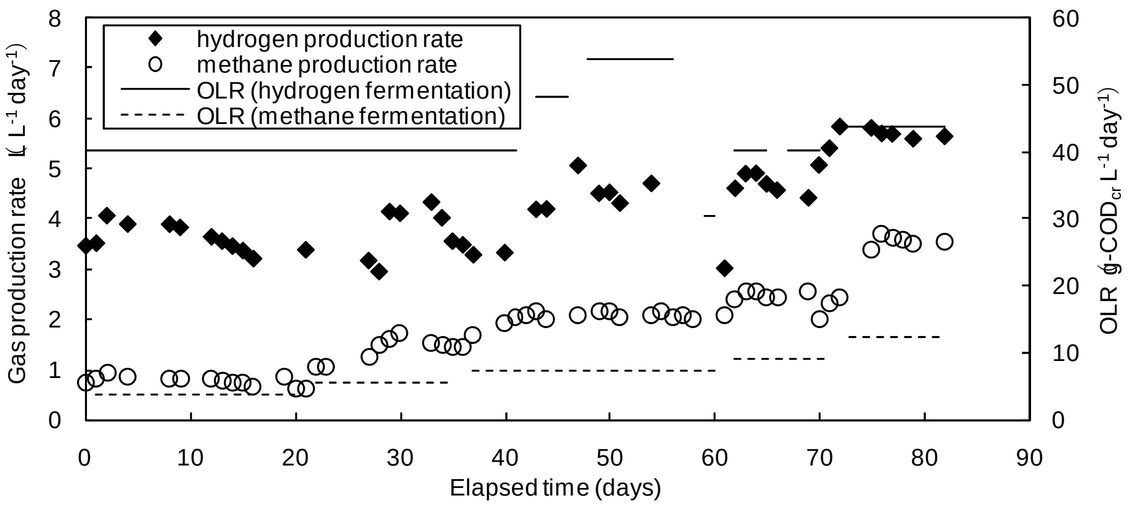


**Figure 3: Time course of gas production in the two-stage continuous fermentation process.**

In the methanogenic process, the initial OLR was set at 3.8 g-COD$_{cr}$ L$^{-1}$ (HRT of 16.3 days), and then gradually raised to 12.4 g-COD$_{cr}$ L$^{-1}$ day$^{-1}$ (HRT of 5.0 days). The $CH_4$ production remained stable around 67-74 L kg$_{AFW}$$^{-1}$ throughout the experiment. The proportions of $CH_4$

and $CO_2$ in the biogas were 68-71 % and 29-32 %, respectively. Table 3 shows the total $COD_{cr}$ removal efficiency and the concentration of volatile fatty acids (VFAs) after the methanogenic process. Although there was no significant difference in the $COD_{cr}$ before and after the hydrogenogenic process, the $COD_{cr}$ removal efficiency reached approximately 80 % after the methanogenic process. Moreover, the concentration of VFAs such as acetate and n-butyrate was much lower after the methanogenic process than after the hydrogenogenic process. This indicates that the VFAs were efficiently converted into $CH_4$ in the methanogenic process.

The average gasification efficiency of the two-stage fermentation process when the OLRs of the $H_2$ and $CH_4$ fermentation were 44.0 and 12.4 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$, respectively, was calculated using the calorific value of the AFW (4031 kJ $kg^{-1}$). The average gasification efficiency in the hydrogenogenic and methanogenic processes was calculated as 10 % and 71 %, respectively. Therefore, the total average gasification efficiency was 81 %.

**Table 3: $COD_{cr}$ removal efficiency and concentration of VFAs in the effluent from $CH_4$ reactor.**

| OLR (g-$COD_{cr}$ $L^{-1}$ $day^{-1}$) | HRT (day) | total $COD_{cr}$ removal (%)* | concentration of VFAs (mg $L^{-1}$) lactate | acetate | propionate | n-butyrate | n-valeriate |
|---|---|---|---|---|---|---|---|
| 3.8 | 16.3 | 81.6 | - | 693 | 160 | - | - |
| 5.6 | 11.1 | 79.6 | - | 1039 | 633 | - | 47 |
| 7.3 | 8.5 | 79.6 | - | 776 | 503 | - | - |
| 9.0 | 6.9 | 81.3 | - | 733 | 377 | - | - |
| 12.4 | 5.0 | 76.9 | - | 351 | 658 | - | - |

*Total $COD_{cr}$ removal (%) = $COD_{cr\ effluent\ from\ methane\ reactor}$ / $COD_{cr\ AFW\ after\ water\ dilution}$ x 100

These results show that the two-stage continuous fermentation process could be operated successfully under all experimental conditions. Further research needs to evaluate the possibility of operation with much higher OLRs in the methanogenic process.

## 4 Conclusions

The performance of a two-stage continuous fermentation process with combined $H_2$ and $CH_4$ reactors was evaluated on a laboratory scale. Artificial food wastes (AFW) were prepared as a culture medium for $H_2$ fermentation. In the experimental optimization of the $H_2$ fermentation, the $H_2$ production in the hydrogenogenic process was stable up to the OLR of 56.1 g-COD $L^{-1}$ $day^{-1}$, and the amount produced was 1.8-2.0 mol-$H_2$ mol-$hexose^{-1}$. In contrast, the $H_2$ production capacity decreased at 67.3g-$COD_{cr}$ $L^{-1}$a $day^{-1}$, suggesting that the fermentation pattern varied along with the increase in lactic acid in the culture medium. In the methanogenic process, the $CH_4$ production was approximately 67-74 L $kg_{AFW}^{-1}$ without any accumulation of VFAs in the $CH_4$ fermentation culture medium throughout the experiment. The average gasification efficiency of the two-stage fermentation process was calculated as 81% when the OLRs of the $H_2$ and $CH_4$ fermentation were 44.0 and 12.4 g-$COD_{cr}$ $L^{-1}$ $day^{-1}$, respectively. These results indicate that the two-stage fermentation process could be operated successfully under these experimental conditions.

**References**

[1] Hawkes, F. R., Dinsdale, R., Hawkes, D. L., Hussy, I. Sustainable fermentative hydrogen production: challenges for process optimisation. *International Journal of Hydrogen Energy*, 2002, 27, 1339-1347

[2] Kraemer, J. T., Bagley, D. M. Continuous fermentative hydrogen production using a two-phase reactor system with recycle. *Environmental Science and Technology*, 2005, 39, 3819-3825.

[3] Kyazze, G., Dinsdale, R., Guwy, A. J., Hawkes, F. R., Premier, G. C., Hawkes, D. L. Performance characteristics of a two-stage dark fermentative system producing hydrogen and methane continuously. *Biotechnology and Bioengineering*, 2006, 97, 759-770.

[4] Kawano, T., Li, YY., Noike, T. Present status and prospective of the study on anaerobic bio-hydrogen (2). *Journal of Water and Waste*, 2005, 47 (11), 39-47.

[5] Takabatake, H., Kohno, Y., Tanno, Y., Noike, T. Influence of pH and metabolites on inhibition of anaerobic hydrogen fermentation by lactic acid bacteria, *Lactobacillus paracasei*. *Environmental Engineering Research.* 2006, 39, 55-65.

[6] Ueno, Y., Kawai, Y., Sato, S., Otsuka, S., Morimoto, M. Biological production of hydrogen from cellulose by natural anaerobic microflora. *Journal of Fermentation and Bioengineering*. 1995, 79, 395-397.